REMARKS

The present invention provides methods for making transformed plants, plant cells, seeds, tissues etc. capable of expressing enzymes of the carotenoid biosynthesis pathway essential to the accumulation of carotenes and/or xanthophylls of interest. The present invention further provides DNA molecules designed to be suitable for producing such plants and plant parts. The currently pending claims relate to the method for making such plants, and to plants made using the method. Claims 1-15, 17-31, and 44-59 are cancelled. Claims 16 and 32-43 are pending and under final rejection. The references cited against the pending claims are listed here:

D1. The Rockefeller Foundation (1993) "Potential for Carotenoid Biosynthesis in Rice Endosperm". International Program on Rice Biotechnology; Workshop Report June 10-11.

D2. Burkhardt et al. (1996) "Genetic Engineering of Provitamin A biosynthesis in Rice Endosperm". Proceedings of the Third International Rice Genetics Symposium IRRI pp 818-820.

D3. Ye *et al.* (2000) "Engineering the Provitamin A (β-carotene) Biosynthetic Pathway into (Carotenoid-Free) Rice Endosperm". Science 287 pp 301-303.

D4. Bramley *et al.* (1997) Pure & Appl. Chem., Vol. 69, No. 10, pp.2159-2162.

In addition, Applicants are submitting herewith, for the Examiner's consideration in connection with the remarks set out below, the following reference:

D5. Potrykus (2001) "Golden Rice and Beyond". Plant Physiology 125 pp.1157-1161.

Claim Rejections Under 35U.S.C. §102

Claims 16 and 32-43 remain rejected under 35 U.S.C. §102(b) as "being anticipated under [D1]". The Examiner disputes our previous assertion that D1 represents early stage discussions and experiments by experts in the field in assessing

the potential for carotenoid production in rice. The Examiner highlights that D1 recites "Amidst optimism that the goal of accumulating nutritionally significant levels of carotenoid synthesis could be achieved the meeting was adjourned".

Two specific "strategies" for providing carotenoid accumulation in the rice endosperm are outlined in D1: (1) a "non-green option" (comprising a bacterial phytoene synthase and a bacterial phytoene desaturase); and (2) a "mixed option" (comprising a plant phytoene synthase and a plant phytoene desaturase). Although D1 does discuss both plant and bacterial genes, it does not specifically disclose a method comprising a combination of a plant phytoene synthase and a bacterial phytoene desaturase. The Examiner has combined separate and distinct elements disclosed in D1 in order to arrive at the present invention, but it is well settled in the law that for a reference to anticipate under 35 U.S.C.§102, every element of the claimed invention must be identically shown in a single reference, and the elements must be arranged as in the claim under review.

Applicants realize that it is also well settled that the legal premise just set out is not "ipsissmis verbis" (is not "expressly... in words"), so that D1 could be considered anticipatory if a person of ordinary skill in the art would understand the reference as disclosing all elements, in proper combination or arrangement, based on their own knowledge and the state of the art. However, in this regard Applicants respectfully draw the Examiner's attention to document D5, submitted herewith, which provides a history of "Golden Rice" (to which the present invention relates). The author of D5 is Professor Ingo Potrykus, who is named as a co-inventor in respect of the present application and who was present at the workshop summarised in D1.

On page 1158 of D5 it is stated "the verdict of this initial session [the Rockefeller workshop] was that such a project had a low probability of success, but that it was worth trying because of its high potential benefit" (emphasis added). Furthermore, it is stated "There are hundreds of scientific reasons why the introduction and coordinated function of these enzymes would not be expected to work. Those with the necessary scientific knowledge were right in not believing in the experiment" (emphasis supplied). Indeed, D1 concludes, *inter alia*, that "it is likely that beta-carotene accumulation in the rice endosperm will require the introduction of

the complete set of genes coding for phytoene synthase onwards" (Appendix D). In addition, D5 highlights the scepticism that existed at the time of D1 concerning the feasibility of successfully engineering an entire biosynthetic pathway into rice. The expectation of success in achieving the desired goal was thus extremely low.

However, contrary to the suggestion of D1, the methods of the present invention do not require a "complete set" of carotenoid biosynthetic genes in order to produce β-carotene in the rice endosperm. Indeed, it came as a surprise that β-carotene, rather than lycopene, accumulation was achieved via the use of only a plant phytoene synthase and a bacterial phytoene desaturase (as evidenced in reference D3, which is discussed further below) in the absence of engineering into the rice plant a lycopene cyclase. Thus, D1 can be seen to teach away from the present invention. Although D1 does recite a plant phytoene synthase and a bacterial phytoene desaturase, it contains no suggestion or motivation to combine these distinct features in order to provide a method to accumulate β-carotene in the rice endosperm.

Thus - although D1 suggests that there may have been "optimism" that significant levels of carotenoid synthesis could be achieved – there actually existed significant doubt among those of skill in the art that a result would actually be achievable. At best, D1 can be seen as an invitation to carry out further research – the reality being that the methods of the present invention for providing β-carotene accumulation in the rice endosperm actually took many years to achieve. This, combined with the fact that D1 does not disclose the claimed combination of a plant phytoene synthase with a bacterial phytoene desaturase, leads to the conclusion that D1 is not anticipatory under 35 U.S.C. §102(b).

The Examiner has also relied on reference D3, Ye et al., in arriving at the conclusion that "The Rockefeller reference teaches inherently, a method of producing plant cells that accumulate carotenoid by transformatino with a gene from daffodil... encoding a phyotene synthase and a gene from daffodil... encoding a phyotene desaturase or by transformation with a gene from daffodil encoding a phytoene synthase and a bacterial *crtI* gene... encoding a phytoene desaturase and plant cells and plants transformed thereby...." However, the foreign priority document to which the present application claims priority was filed on March 5, 1999,

and D3 was published in the 14 January 2000 issue of Science. Furthermore, D3 represents the work of Beyer and Potrykus as disclosed in the present application, and thus could not have appeared before the making of the claimed invention. In any event, the publication of D3 occurred well after the priority date, and less than one year from the §371 international filing date (March 3, 2000), and therefore D3 is not available as a reference under either 35 U.S.C.§102(a) or (b).

Therefore, for the foregoing reasons it is respectfully submitted that the subject matter of the presently submitted claims is in fact novel over D1, and Applicants respectfully request that this rejection be withdrawn.

Claim Rejections Under U.S.C §103

Claims 16 and 32-43 are rejected under 35 U.S.C.§103 as allegedly being obvious over D2 and D4. The Examiner suggests that, in view of D2 and D4, "one would have had reasonable expectation of success in transforming a rice plant with a plant phytoene synthase and a bacterial phytoene desaturase; and in producing provitamin A or beta carotene in the endosperm of rice". Respectfully, Applicants strongly disagree with this assertion, for the following reasons.

As discussed above with regard to D1, D2 suggests (page 819) that four enzymes are necessary for β-carotene biosynthesis in the rice endosperm (phytoene synthase, phytoene desaturase, ζ-carotene desaturase and lycopene cyclase). Thus D2, like D1, teaches away from the claimed method for reasons already stated above.

D2 also discloses a transgenic rice plant purported to comprise a plant (daffodil) phytoene synthase and a plant (daffodil) phytoene desaturase. It is reported that the transgenic rice plant accumulated a single carotenoid (phytoene) but not ζ-carotene, the product of phytoene desaturase (page 820).

The work reported in D2 was important since it indicated that was possible to produce phytoene in the rice endosperm (indeed this was considered to be the "first breakthrough" in the development of Golden Rice - D5 page1158). However, D2 does

not solve the problem of the production of carotenoids other than phytoene in the rice endosperm – since the phytoene desaturase that was introduced did not result in the accumulation of ζ-carotene as expected. Furthermore, D2 does not teach, or even suggest, a method by which β-carotene can be produced in the rice endosperm. There is certainly no suggestion in D2 that β-carotene can be produced in the rice endosperm by replacing the plant phytoene desaturase with a bacterial phytoene desaturase. Indeed, the skilled person would understand from D1 that the use of bacterial carotenoid gene(s) to produce carotenoids in plants is not ideal – since, while they appear to be functional, they can result in non-viable plants (D1 - page 3).

Bramley *et al.* (D4) disclose, *inter alia*, the use of a bacterial phytoene desaturase gene (*crt* I) to increase carotenoid levels in tissues (tomato fruit) which already have the capacity to synthesis high levels of carotenoids. Thus the skilled person would appreciate that, in the methods taught by Bramley, all of the biosynthetic machinery required to produce carotenoids are already present in the tissues transformed with the *crt*I gene. D4 is thus not concerned with the problem addressed by present invention – that is the accumulation of carotenoids in tissues which are normally carotenoid free.

There is no suggestion in either D2 or D4 that β-carotene accumulation could be achieved in the rice endosperm by the use of a plant phytoene synthase and a bacterial phytoene desaturase (e.g *crt* I) alone. Although it is possible to combine the teachings of D2 and D4 and arrive at the present invention there is absolutely no motivation to do so in the absence of the present invention. In doing so, it is submitted that the Examiner has made an *ex-post facto* analysis based on hingsight. Therefore, Applicants respectfully submit that the present rejection has been addressed, and should be withdrawn.

CONCLUSION

Applicants respectfully request reconsideration of the claims on the merits in light of the foregoing remarks, and allowance of the pending claims. Applicants are also filing herewith a notice of appeal. Should the Examiner consider a telephone call

to the attorney for Applicants to be helpful in progressing the case to allowance one is earnestly requested.

Respectfully submitted,

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